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Association between zidovudine-containing antiretroviral therapy exposure *in utero* and leukocyte telomere length at birth

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Abstract

Objectives: Zidovudine (ZDV) is a nucleoside reverse transcriptase inhibitor that could cause telomere shortening through inhibition of telomerase. We examined the association between *in utero* exposure to ZDV and telomere length (TL) at birth in HIV-exposed uninfected (HEU) newborns.

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POTENTIAL CONFLICTS OF INTEREST

All authors: No potential conflicts of interest

Methods: We selected 94 ZDV-exposed HEU children and 85 antiretroviral therapy (ART)-unexposed HEU children from the Surveillance Monitoring for ART Toxicities Study and the Women and Infants Transmission Study. We assessed relative TL in stored peripheral blood mononuclear cells taken in the first 7 days of life using quantitative polymerase chain reaction. We used linear regression to compare relative TL between ZDV-exposed and ART-unexposed children. We additionally evaluated relative TL according to maternal and infant characteristics.

Results: Relative TL was longer in ZDV-exposed children compared to ART-unexposed individuals (adjusted mean ratio difference 0.21, 95%CI 0.15–0.28, p<0.001). We found an inverse correlation between maternal HIV RNA levels and infant relative TL (-0.06 per $\log_{10}$ copies, 95%CI −0.08 to −0.03, p<0.001). Relative TL was not associated with maternal CD4 count, maternal age, gestational age, sex, sample storage time, or maternal substance use (p>0.05).

Conclusion: Relative TL was longer in ZDV-exposed infants. This difference may reflect beneficial health effects of antiretroviral therapy during pregnancy, since we observed an inverse association with maternal HIV RNA levels.

Keywords

HIV-exposed uninfected; pregnancy; zidovudine; nucleoside reverse transcriptase inhibitor; telomere length

INTRODUCTION

Antiretroviral therapy (ART) has dramatically reduced AIDS morbidity and mortality as well as mother-to-child transmission of HIV. Zidovudine (ZDV) was introduced in 1994 to prevent vertical transmission and has been widely used [1], usually with other antiretrovirals. In the US as of 2009, 73% of HIV-exposed but uninfected (HEU) children (born to HIV-infected mothers) were exposed *in utero* to ZDV [2].

ZDV is a nucleoside reverse transcriptase inhibitor (NRTI) with antiretroviral effects that include competitive inhibition of nucleoside binding to HIV reverse transcriptase and DNA chain-termination. Carcinogenic properties of ZDV have been suggested based on *in vitro* and animal studies (summarized in [3]). In response to existing evidence, the International Agency for Research on Cancer classified ZDV as possibly carcinogenic to humans [3].

Telomeres are nucleoprotein complexes located at chromosome ends that protect chromosome integrity. Telomeres shorten with each cell division due to incomplete replication of the 3' DNA end. Telomerase is a reverse transcriptase that extends the $TTTAGG_{(n)}$ nucleotide repeats at chromosome ends. Since NRTIs including ZDV are known to inhibit telomerase activity *in vitro* [4, 5], it has been postulated that telomere length (TL) in individuals taking ZDV could be shorter than normal. Shorter TL after ZDV exposure has been observed *in vitro* and in animal studies [6, 7].

It is important to determine whether NRTIs affect TL, because abnormal TL is implicated in diseases including both cancer [8] and cardiovascular disease [9]. In HIV infected individuals, ART including NRTIs has been shown to increase mean blood TL [10, 11]. However, data on HEU children after *in utero* NRTI exposure are limited. A prior epidemiologic study of HEU

children found no association between leukocyte TL and *in utero* ART exposure (predominately ZDV-based regimens) [12], while another study showed longer leukocyte TL in HEU children exposed to ZDV, lamivudine, and nevirapine or nelfinavir than in HIV-unexposed uninfected children [13]. However, these studies were limited by a small number of ART-unexposed controls (n=39) [12] or lack of comparison with ART-unexposed HEU children [13]. In the current study, we examine the association between *in utero* exposure to ZDV and peripheral blood mononuclear cell TL in newborn HEU children.

METHODS

This study included data and specimens from 179 HEU children; 94 were exposed to ZDV *in utero* (most of whom were also exposed to other ART medications), and 85 were unexposed to ZDV or other ART medications. ZDV-exposed children were born during 2007–2017 (except one in 1995), and ART-unexposed children during 1990–1996 (except one in 2017). ZDV-exposed children were selected from the Surveillance Monitoring for ART Toxicities (SMARTT) Study as part of the Pediatric HIV/AIDS Cohort Study network, which has enrolled children with detailed *in utero* ART exposure data into two cohorts from 22 sites in the US and Puerto Rico; its Static cohort includes HEU children born as early as 1995 and the Dynamic cohort includes HEU children born in 2007 through the present [14, 15]. ART-unexposed children were mostly from the Women and Infants Transmission Study (WITS, n=84; n=1 from SMARTT), which enrolled pregnant women living with HIV and their children from 6 US sites during 1989–2003 [16]. Adopting a previously applied method [17], we used a historical comparison group of ART-unexposed children, because a contemporaneous unexposed group in the current ART era in the US would oversample children whose mothers were not receiving proper medical care. Children were frequency-matched on sex, race, and maternal substance use.

We retrieved information on maternal ART during pregnancy and participant characteristics at birth including maternal age, gestational age, birthweight, and latest maternal HIV RNA levels and CD4 cell counts before delivery. Information on women's race/ethnicity, smoking, alcohol consumption, and illicit substance use was obtained by self-report. All mothers provided written informed consent for enrollment into SMARTT and/or WITS. The present study used deidentified samples and was exempted from human subjects review by the Office of Human Subjects Research Protections at the National Institutes of Health.

Peripheral blood mononuclear cells (PBMCs) were obtained in the first 7 days of life and frozen. DNA was extracted in a single batch using the same method. TL was measured using quantitative polymerase chain reaction (qPCR), adapted from [18] and described in [19]. Briefly, the ratio of amplified signals for telomere (T) and an autosomal single copy gene *36B4* (S) was normalized to internal quality control samples to yield a standardized T/S ratio (hereafter referred to as "relative TL"). All samples were assayed in triplicate and average values were used for statistical analyses. The coefficient of variation for control samples across the three plates was 4.9%.

We compared demographic and clinical characteristics between ZDV-exposed and ART-unexposed children using chi-square, Fisher exact, and Wilcoxon rank sum tests. Linear

regression was used to evaluate the association between ZDV and relative TL and a stepwise method for covariate selection was used in adjusted models (p=0.15 for entry and exit). Statistical analyses were performed using SAS version 9.4 or R version 3.4.4. Statistical significance was defined using two-sided p<0.05.

RESULTS

Compared with ART-unexposed children, ZDV-exposed children had lower gestational age at birth (median, 38.2 *versus* 39 weeks, p<0.001). Mothers of ZDV-exposed children were older at delivery (median, 28.5 *versus* 26.0 years, p=0.003) and were more likely to have suppressed HIV RNA < 400 copies/ml (88% *versus* 12%, p<0.001) than mothers of ART-unexposed children. Maternal tobacco, alcohol and marijuana use (no mothers used illicit drugs other than marijuana) during pregnancy did not differ (Table 1). Among ZDV-exposed children, 78% were exposed to ZDV during the first trimester, and all were exposed during the second and third trimesters (median duration 29.4 weeks). About 88% of the ZDV-exposed children were also exposed to other antiretrovirals (8.5% non-nucleoside reverse transcriptase inhibitors [NNRTIs] but not protease inhibitors [PIs], 63.8% PIs but not NNRTIs, 10.6% to both NNRTIs and PIs). About 98% of ZDV-exposed children were also exposed to other NRITs (Supplemental Table 1). The most frequent concomitant NRTI used was lamivudine (90.4%), followed by tenofovir (33.0%), emtricitabine (28.7%) and abacavir (18.1%).

ZDV exposure was associated with longer relative TL at birth (mean±standard deviation T/S, 0.85±0.23 *versus* 0.65±0.19, p<0.001) (Figure 1A). After adjusting for race, ZDV exposure remained statistically significantly associated with longer relative TL (adjusted mean T/S difference, 0.21, 95%CI 0.15–0.28, p<0.001). Further adjustment for gestational age and birth weight did not change the result (adjusted mean T/S difference, 0.21, 95%CI 0.14–0.27, p<0.001). Among ZDV-exposed children, the duration of ZDV exposure was not associated with relative TL (p=0.45, Supplemental Figure 1). In addition, no statistically significant differences were noted between children exposed to ZDV and PIs and those exposed to ZDV without PIs (p=0.08), or between children exposed to ZDV and NNRTIs and those exposed to ZDV without NNRTIs (p=0.33) (Supplemental Figure 2A–B).

Relative TL was inversely associated with maternal HIV RNA (-0.06 per $\log_{10}$ copies, 95%CI −0.08 to −0.03, p<0.001, Figure 1B). In ZDV exposure stratified analyses, relative TL was not associated with maternal HIV RNA levels in ZDV-exposed (0.04 per $\log_{10}$ copies, p=0.20, Figure 1C) or ART-unexposed children (-0.02 per $\log_{10}$ copies, p=0.18). Relative TL was not associated with maternal CD4 count or other characteristics including maternal age, gestational age, sex, sample storage time, or maternal substance use (Supplemental Figure 3A–H).

DISCUSSION

In this study of 179 newborn HEU children, *in utero* ZDV exposure was associated with longer relative TL in PBMC. Our results differ from a previous study that found no association between *in utero* ART exposure and TL [12]. However, that study included few

ART-unexposed children. Moreover, ART exposure was shorter in that study (median 20 *versus* 29.4 weeks in our study) with higher maternal viral loads close to delivery among ART-exposed children (median 1455 *versus* 48 copies/mL in our study) [12], suggesting that suboptimal viral suppression or a shorter duration of reverse transcriptase inhibition may have influenced the study results.

We found that maternal HIV RNA levels were inversely correlated with infant TL, suggesting effective treatment of HIV contributes to the longer TL in ZDV-exposed children. Longer TL in ZDV-exposed children could be related to better maternal health and more favorable gestational growth environment. Given our modest sample size, we cannot rule out that the longer TLs were present only for a subgroup of ZDV-exposed children related to use of certain other antiretroviral medications as combination ART was common in the ZDV-exposed group (Table 1). It is also possible that ZDV could have had an adverse effect on telomerase and infant TL that was masked by other beneficial effects of ART. Maternal HIV viral load has been associated with changes in CD8 T-cells subsets in HEU children [20]. However, it is unclear whether alterations in T-cell subpopulations could explain our results, and we did not have TL data on leukocyte subtypes to evaluate this hypothesis. Considering the short exposure period (≤7 days) prior to our assessment of TL, we do not expect that ZDV prophylaxis during or after birth to impact TL in HEU children in our study. Not surprisingly, it has been shown that leukocyte TL in HEU newborns is not affected by ZDV prophylaxis [13].

A strength of our study is the use of a control group comprising HEU children without any ART exposure (including NRTIs) *in utero*. Secondly, matching children on potential confounders reduced the possibility that those influenced our results. Our study also has limitations. Although we selected children matching on important confounders, unmeasured factors may have influenced our findings. Given that ART-unexposed children were born earlier than ZDV-exposed children, differences in sample storage time or advances in prenatal care over time could have played a role in the observed TL differences. There has also been growing use of other NRTIs over time, and these may have different *in utero* effects on TL. Additionally, qPCR measurement of relative TL is influenced by laboratory factors [21]. However, DNA in this study was extracted from frozen PBMCs in a single batch using the same method. Lastly, due to the small sample size of our study, we were limited in our ability to examine subgroups.

In conclusion, we observed that ZDV use during pregnancy was associated with longer relative TL at birth relative to ART-unexposed HEU children. Future studies are needed to elucidate biological mechanisms linking *in utero* antiretroviral exposure to PBMC TL in newborns.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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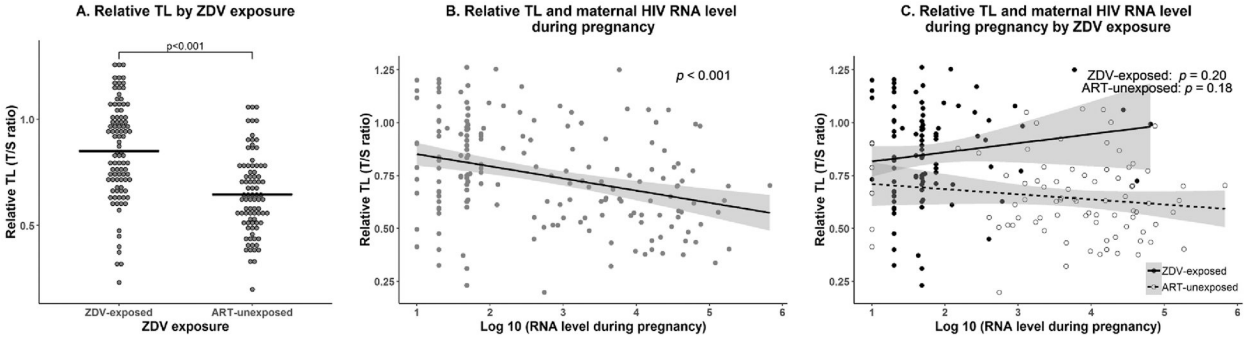

Figure 1. Association of relative telomere length at birth with zidovudine exposure *in utero* and mother's HIV RNA level during pregnancy

a. Associations between relative telomere length at birth and zidovudine exposure *in utero*. Horizontal bars denote mean relative telomere length values.

b. Associations between relative telomere length at birth and mother's last HIV RNA level during pregnancy for all subjects. RNA level data were $\log_{10}$ transformed.

c. Associations between relative telomere length at birth and mother's last HIV RNA level during pregnancy according to *in utero* zidovudine exposure status. RNA level data were $\log_{10}$ transformed. Closed circle/solid line: ZDV-exposed, open circle/dotted line: ART-unexposed.

Table 1.

Participant characteristics

Variable	ZDV-exposed (N=94)		ART-unexposed (N=85)		p
Birth weight (kg), median (range)	3.0	(1.7, 4.4)	3.1	(1.9, 4.6)	0.026[1]
Birth weight, n (%)					0.24[2]
1,500–2,500 gm	13	(14%)	7	(8%)	
≥ 2,500 gm	81	(86%)	78	(92%)	
Gestational age (weeks), median (range)	38.2	(32.6, 42.1)	39.0	(32.0, 43.0)	<0.001[1]
Gestational age, n (%)					0.31[3]
32 to <34 weeks	1	(1%)	1	(1%)	
34 to <37 weeks	16	(17%)	8	(9%)	
37 weeks or more	77	(82%)	76	(89%)	
Sex, n (%)					0.85[2]
Male	50	(53%)	44	(52%)	
Female	44	(47%)	41	(48%)	
Group, n (%)					<0.001[3]
Public WITS	0	(0%)	84	(99%)	
SMARTT WITS	1	(1%)	0	(0%)	
SMARTT w/o WITS	93	(99%)	1	(1%)	
Year of birth, n (%)					<0.001[2]
1990–1994	0	(0%)	82	(96%)	
1995–2017	94	(100%)	3	(4%)	
Mothers age at delivery (years), median (range)	28.5	(16.9, 45.0)	26.0	(15.0, 40.0)	0.003[1]
Participant's race/ethnicity, n (%)					0.01[3]
White	6	(6%)	13	(15%)	
African American	33	(35%)	37	(44%)	
Hispanic	53	(56%)	29	(34%)	
Other	2	(2%)	6	(7%)	
Days from birth to sample draw, median (range)	1.0	(0, 7.0)	1.0	(0, 6.9)	0.76[1]
Last CD4 count during pregnancy (cells/mm^3), median (range)	502.0	(34.0, 1198.0)	595.0	(74.0, 2330.0)	0.07[1]
Last RNA level during pregnancy (copies/ml), median (range)	48	(0, 64361)	8079	(0, 672005)	<0.001[1]
<400	82	(88%)	10	(12%)	<0.001[2]
≥400	11	(12%)	75	(88%)	
Mother ever used tobacco, n (%)					0.27[2]
Yes	26	(28%)	30	(35%)	
No	68	(72%)	55	(65%)	
Mother ever used alcohol, n (%)					0.11[2]
Yes	24	(26%)	31	(36%)	

Variable	ZDV-exposed (N=94)		ART-unexposed (N=85)		p
No	70	(74%)	54	(64%)	
Mother ever used marijuana/hashish, n (%)					0.19[2]
Yes	15	(16%)	8	(9%)	
No	79	(84%)	77	(91%)	
NNRTIs use during pregnancy, n (%)					<0.001[2]
Yes	18	(19%)	0	(0%)	
No	76	(81%)	85	(100%)	
PIs use during pregnancy, n (%)					<0.001[2]
Yes	70	(74%)	0	(0%)	
No	24	(26%)	85	(100%)	

1. Wilcoxon rank-sum test

2. Chi-square test

3. Fisher's exact test

Abbreviations: ZDV: zidovudine; ART: antiretroviral therapy; WITS: The Women and Infants Transmission Study; SMARTT: The Surveillance Monitoring for ART Toxicities Study; NNRTIs: non-nucleoside reverse transcriptase inhibitors; PIs: protease inhibitors